| | |
|---|---|
| L-Cysteine | 0.8 |
| L-Glutamate | 2.1 |
| L-Glutamine | 0.7 |
| Glycine | 2.7 |
| L-Histidine | 0.3 |
| L-Isoleucine | 0.8 |
| L-Leucine | 0.8 |
| L-Lysine-HCl | 1.4 |
| L-Methionine | 0.7 |
| L-Phenylalanine | 1.2 |
| L-Proline | 2.6 |
| L-Serine | 2.9 |
| L-Threonine | 1.7 |
| L-Tryptophan | 0.5 |
| L-Tyrosine | 0.3 |
| L-Valine | 0.9 |
| $K_2SO_4$ | 0.28[a] |
| $KH_2PO_4/K_2HPO_4$ | 4/6 |
| Na-acetate | 15 |
| $CaCl_2$ | 0.0005[a] |
| $MgCl_2$ | 0.52[a] |
| $FeSO_4$ | 0.01[a] |
| Vitamins[b] | + |
| Micronutrients[a,c] | + |
| Citric acid | 0.1 |

B2 cont'd

[a] From Neidhardt et al. J. Bacteriol. **119**:736-747;

[b] Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

[c] Micronutrients: 0.003 μM $(NH_4)_6(MO_7)_{24}$, 0.4 μM $H_3BO_4$, 0.03 μM $CoCl_2$, 0.01 μM $CuSO_4$, 0.08 μM $MnCl_2$ and 0.01 μM $ZnSO_4$.

Please add new claims 25-29 as follows:

--25. (new) A method according to claim 12 wherein the chemically defined medium is the medium comprising:

B3

| Component | Concentration, mM or +/- |
|---|---|
| L-Alanine | 3.4 |
| L-Arginine | 1.1 |
| L-Asparagine | 0.8 |
| L-Cysteine | 0.8 |
| L-Glutamate | 2.1 |
| L-Glutamine | 0.7 |
| Glycine | 2.7 |

| | |
|---|---|
| L-Histidine | 0.3 |
| L-Isoleucine | 0.8 |
| L-Leucine | 0.8 |
| L-Lysine-HCl | 1.4 |
| L-Methionine | 0.7 |
| L-Phenylalanine | 1.2 |
| L-Proline | 2.6 |
| L-Serine | 2.9 |
| L-Threonine | 1.7 |
| L-Tryptophan | 0.5 |
| L-Tyrosine | 0.3 |
| L-Valine | 0.9 |
| $K_2SO_4$ | 0.28[a] |
| $KH_2PO_4/K_2HPO_4$ | 4/6 |
| Na-acetate | 15 |
| $CaCl_2$ | 0.0005[a] |
| $MgCl_2$ | 0.52[a] |
| $FeSO_4$ | 0.01[a] |
| Vitamins[b] | + |
| Micronutrients[a,c] | + |
| Citric acid | 0.1 |

[a] From Neidhardt et al. J. Bacteriol. **119**:736-747;

[b] Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

[c] Micronutrients: 0.003 μM $(NH_4)_6(MO_7)_{24}$, 0.4 μM $H_3BO_4$, 0.03 μM $CoCl_2$, 0.01 μM $CuSO_4$, 0.08 μM $MnCl_2$ and 0.01 μM $ZnSO_4$;

wherein the components of said chemically defined medium are present in three-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

26. (new) A method according to claim 12 wherein the chemically defined medium is the medium comprising:

| Component | Concentration, mM or +/- |
|---|---|
| L-Alanine | 3.4 |
| L-Arginine | 1.1 |
| L-Asparagine | 0.8 |
| L-Cysteine | 0.8 |
| L-Glutamate | 2.1 |
| L-Glutamine | 0.7 |
| Glycine | 2.7 |
| L-Histidine | 0.3 |

| | |
|---|---|
| L-Isoleucine | 0.8 |
| L-Leucine | 0.8 |
| L-Lysine-HCl | 1.4 |
| L-Methionine | 0.7 |
| L-Phenylalanine | 1.2 |
| L-Proline | 2.6 |
| L-Serine | 2.9 |
| L-Threonine | 1.7 |
| L-Tryptophan | 0.5 |
| L-Tyrosine | 0.3 |
| L-Valine | 0.9 |
| $K_2SO_4$ | 0.28[a] |
| $KH_2PO_4/K_2HPO_4$ | 4/6 |
| Na-acetate | 15 |
| $CaCl_2$ | 0.0005[a] |
| $MgCl_2$ | 0.52[a] |
| $FeSO_4$ | 0.01[a] |
| Vitamins[b] | + |
| Micronutrients[a,c] | + |
| Citric acid | 0.1 |

[a] From Neidhardt et al. J. Bacteriol. **119**:736-747;

[b] Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

[c] Micronutrients: 0.003 μM $(NH_4)_6(MO_7)_{24}$, 0.4 μM $H_3BO_4$, 0.03 μM $CoCl_2$, 0.01 μM $CuSO_4$, 0.08 μM $MnCl_2$ and 0.01 μM $ZnSO_4$;

wherein the components of said chemically defined medium are present in five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

27. (new) A method according to claim 12 wherein the chemically defined medium is the medium comprising:

| Component | Concentration, mM or +/- |
|---|---|
| L-Alanine | 3.4 |
| L-Arginine | 1.1 |
| L-Asparagine | 0.8 |
| L-Cysteine | 0.8 |
| L-Glutamate | 2.1 |
| L-Glutamine | 0.7 |
| Glycine | 2.7 |
| L-Histidine | 0.3 |
| L-Isoleucine | 0.8 |

| | |
|---|---|
| L-Leucine | 0.8 |
| L-Lysine-HCl | 1.4 |
| L-Methionine | 0.7 |
| L-Phenylalanine | 1.2 |
| L-Proline | 2.6 |
| L-Serine | 2.9 |
| L-Threonine | 1.7 |
| L-Tryptophan | 0.5 |
| L-Tyrosine | 0.3 |
| L-Valine | 0.9 |
| $K_2SO_4$ | 0.28[a] |
| $KH_2PO_4/K_2HPO_4$ | 4/6 |
| Na-acetate | 15 |
| $CaCl_2$ | 0.0005[a] |
| $MgCl_2$ | 0.52[a] |
| $FeSO_4$ | 0.01[a] |
| Vitamins[b] | + |
| Micronutrients[a,c] | + |
| Citric acid | 0.1 |

[a] From Neidhardt et al. J. Bacteriol. **119**:736-747;

[b] Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

[c] Micronutrients: 0.003 μM $(NH_4)_6(MO_7)_{24}$, 0.4 μM $H_3BO_4$, 0.03 μM $CoCl_2$, 0.01 μM $CuSO_4$, 0.08 μM $MnCl_2$ and 0.01 μM $ZnSO_4$;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L.

28. (new) A method according to claim 12 wherein the chemically defined medium is the medium comprising:

| Component | Concentration, mM or +/- |
|---|---|
| L-Alanine | 3.4 |
| L-Arginine | 1.1 |
| L-Asparagine | 0.8 |
| L-Cysteine | 0.8 |
| L-Glutamate | 2.1 |
| L-Glutamine | 0.7 |
| Glycine | 2.7 |
| L-Histidine | 0.3 |
| L-Isoleucine | 0.8 |
| L-Leucine | 0.8 |
| L-Lysine-HCl | 1.4 |

| | |
|---|---|
| L-Methionine | 0.7 |
| L-Phenylalanine | 1.2 |
| L-Proline | 2.6 |
| L-Serine | 2.9 |
| L-Threonine | 1.7 |
| L-Tryptophan | 0.5 |
| L-Tyrosine | 0.3 |
| L-Valine | 0.9 |
| $K_2SO_4$ | 0.28[a] |
| $KH_2PO_4/K_2HPO_4$ | 4/6 |
| Na-acetate | 15 |
| $CaCl_2$ | 0.0005[a] |
| $MgCl_2$ | 0.52[a] |
| $FeSO_4$ | 0.01[a] |
| Vitamins[b] | + |
| Micronutrients[a,c] | + |
| Citric acid | 0.1 |

[a] From Neidhardt et al. J. Bacteriol. **119**:736-747;

[b] Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

[c] Micronutrients: 0.003 μM $(NH_4)_6(MO_7)_{24}$, 0.4 μM $H_3BO_4$, 0.03 μM $CoCl_2$, 0.01 μM $CuSO_4$, 0.08 μM $MnCl_2$ and 0.01 μM $ZnSO_4$;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L, and the components of said chemically defined medium are present in three-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

29. (new) A method according to claim 12 wherein the chemically defined medium is the medium comprising:

| Component | Concentration, mM or +/- |
|---|---|
| L-Alanine | 3.4 |
| L-Arginine | 1.1 |
| L-Asparagine | 0.8 |
| L-Cysteine | 0.8 |
| L-Glutamate | 2.1 |
| L-Glutamine | 0.7 |
| Glycine | 2.7 |
| L-Histidine | 0.3 |
| L-Isoleucine | 0.8 |
| L-Leucine | 0.8 |
| L-Lysine-HCl | 1.4 |